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# BIOLOGICAL BULLETIN

## THE NATURE OF THE FERTILIZATION MEMBRANE OF *ASTERIAS* AND *ARBACIA* EGGS.[1]

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It is generally believed that the fertilization membrane of an echinoderm egg is a distinct structure formed by the egg, and separated from it by the liquid filled perivitelline space. Views quite at variance with this current one have been advanced in recent communications; these we believe are not justified by the experimental evidence which is to be considered below.

I. Elder,[2] Kite[3] and McClendon[4] have separately maintained that the egg jelly is essential to the formation of the fertilization membrane and in one way or another enters into its composition. Harvey[5] has made this question one of special study and presents convincing experimental evidence to show that the egg jelly is not essential. He was able by repeated shaking and continued washing with sea water, to remove this jelly so completely that not a trace could be detected when the eggs were examined in India ink suspensions. Fertilizations of such eggs caused the formation of fertilization membranes, which however may be more tenuous than normally, probably due to the fact that the jelly facilitates the membrane formation by acting as a mechanical block to the diffusion of the membrane forming colloid ("membranogen") which was thus retained in more concentrated solution and consequently formed a denser membrane.

J. Loeb[6] found that hydrochloric acid would dissolve the jelly

[1] From the Physiological Laboratory of Tulane University, New Orleans, and the Marine Biological Laboratory, Woods Hole.

[2] Elder, *Arch. f. Entwick.*, 1913, XXXV., 195.

[3] Kite, G. L., *Science*, 1912, N.S., XXXVI., 562.

[4] McClendon, J. F., *Internat. Zeitschr. f. physik. chem. Biol.*, 1914, I., 163.

[5] Harvey, E. N., BIOL. BULL., 1914, XXVII., 237.

[6] Loeb, J., *Science*, 1914, N.S., XL., 318.

from the eggs of *Strongylocentrotus purpuratus* and that after subsequent treatment with sodium hydroxide and calcium chloride, starfish sperm caused the formation of fertilization membranes and induced development. F. R. Lillie[1] found that *Arbacia* "eggs are fertilizable" after complete removal of the jelly by Loeb's method. Loeb and Kupelwieser[2] caused the formation of fertilization membranes by treating eggs with butyric acid; these were freed from every vestige of membrane and jelly was removed by shaking, yet upon insemination they were able to form new fertilization membranes. Moore[3] noted that egg fragments, produced by shaking, formed membranes upon entrance of the spermatozoön. These observations indicate that the egg jelly is not essential for the formation of the fertilization membrane.

II. The facts considered in the preceding section make us doubt the validity of the hypothesis advanced by McClendon that the fertilization membrane is formed by the process of precipitation due to the contact of two colloids carrying opposite electric charges; viz., the egg jelly which McClendon states is electro-negative and another colloid (membranogen) derived from the egg which he found to be electropositive. This view is rendered quite untenable when we consider the fact that fertilization membranes are formed about echinoderm eggs by Loeb's well-known methods of inducing artificial parthenogenesis by treatment with weak fatty acids. These acids act only after penetrating the egg-jelly in the course of which event they impart to that colloid the positive electric charge of the dominant hydrogen ion; they therefore have the same charge as the colloids of the egg and the requisite conditions for precipitation of colloids do not exist.

It has furthermore been shown that in the chemical fertilization of *Asterias* eggs either acids or alkalies may be used to induce membrane formation. The electric charge of the jelly is positive when acids are used and negative with alkalis; obviously the charge is not of opposite sign to that of the egg substance (membranogen) in both cases.

[1] Lillie, F. R., BIOL. BULL., 1915, XXVIII., 24.

[2] Kupfelweiser, H., *Arch. f. Entw.*, 1909, XXVII., 434.

[3] Moore, A. R., Univ. California Pub., 1912, IV., 89.

Upon treatment with acids the egg jelly swells perceptibly and also removes the acid from solution either by adsorbing it or by chemically uniting with it. In either event the jelly becomes electropositive. The fixation of the acid is shown in the following experiments.

Ripe eggs from *Asterias* ovaries are shed in sea water and allowed to settle to the bottom of a graduated cylinder. The supernatant liquid is decanted and enough fresh sea water added to the mass of eggs to equal three times their volume. The cylinder is actively shaken and the eggs again are allowed to settle. The clear supernatant fluid containing a considerable amount of the jelly is now decanted off and an equal volume of $N/500$ butyric acid added. Fresh *Asterias* egg do not form fertilization membranes in this acid-jelly mixture but do so in a control solution of $N/1{,}000$ butyric acid in sea water. On the other hand fertilization membranes are formed at once in the acid-jelly mixture after the further addition of an equal volume of $N/500$ butyric acid sea water, thus indicating clearly that the initial effect of the jelly is to remove the acid from solution and that membrane formation is induced only when acid is present in excess of the amount necessary to saturate the jelly. Neglect in the observance of this precaution doubtless has been the cause of many failures to obtain good artificial fertilization by this method in case large masses of eggs have been added to small amounts of acid sea water. Loeb[1] cautioned against such a loose procedure. Under such conditions the relatively large amount of adherent jelly fixes the acid and prevents it from acting on the eggs.

III. The formation of fertilization membranes after the complete removal of the jelly indicates the origin of this structure from the egg. The following experiment furnishes a simple but equally conclusive mechanical demonstration of this fact. Fertilization of the eggs of *Asterias* either by sperm or by artificial means such as acid or heat (30–33° C.) is possible not only after maturation but also earlier, when the nuclear membrane about the germinal vesicle just begins to fade (De Lage[2]); in fact I have

[1] Loeb, J., "Artificial Parthenogenesis" (Chicago), 1913, 69.

[2] DeLage, Y., *Arch. de zoöl. expér. et gen.*, ser. 3, IX., 285.

often observed that fertilization is possible even before the nuclear membrane begins to fade. After both polar bodies were formed I found that fertilization by any means caused the formation of the membrane between them and the egg surface, from which they were lifted and pushed away as the membrane moved outward. They are seen to lie in the saucer-like depression in the outer surface of the membrane, which results from the resistance offered by the egg-jelly to the pressure developed in the perivitelline space. After staining with dilute methylene blue, examination removes all doubt that the polar bodies are entirely outside the membrane.

By so timing the fertilization that only the first polar body has been formed, it will be pushed away from the egg outside the fertilization membrane while the second polar body, being formed much later, remains at the surface of the egg, and therefore inside the perivitelline space. Similarly by fertilizing before maturation the fertilization membrane will have been formed and will have left the surface of the egg before the polar bodies are extruded. These remain at the surface of the egg and therefore within the perivitelline space. By manipulation they may be shaken free from the surface of the egg and moved about freely.

IV. Kite[1] has stated that the "so-called fertilization membrane of the egg of *Arbacia* consists of three parts, viz., the inner layer of the egg jelly which has undergone a change in refraction index, the swollen vitelline membrane, and the thin highly refractive surface layer of the cytoplasm. This hyaline layer is still very adherent to the vitelline membrane." Kite thus conceives the vitelline membrane to remain attached to the egg, to become swollen and edematous and to fill completely the entire space between the egg and the jelly which in turn is altered in refractive power to appear like a separate membrane. That the egg jelly is non-essential has been shown. The other features of Kite's conception can likewise be demonstrated to be erroneous both for *Arbacia* and *Asterias*. In these forms what appears to be the fertilization membrane is far more than the outer refractive part of an invisible thick layer of "gel" formed by a swelling of the vitelline membrane. It is a true, thin membrane formed

[1] Kite, G. L., *Science*, 1912, N.S., XXXVI., 562.

by the egg and separated from its surface by a liquid filled space as is indicated by the following considerations:

1. This new structure has entirely different permeability from that of the surfaces of either the fertilized or unfertilized eggs which are very slightly permeable to neutral salts, so that the eggs are plasmolyzed and crenated by hypertonic saline solutions. Salts however must penetrate the fertilization membrane freely to effect this change in the egg and that they do so is further shown by the fact that the fertilization membrane retains its round contour and distended condition when placed in the hypertonic or hypo-tonic salt solutions. The differences in permeability thus far demonstrated speak against the origin of the membrane from a pre-existing structure on the surface of the egg.

2. Kite's description is proven incorrect by the demonstration of the fact that the fertilization membrane is separated from the surface of the egg by a space filled with liquid and not occupied by a swollen gelatinous vitelline membrane continuous with the surface of the egg on the inside and with the egg jelly on the outside. Dr. Robt. Chambers by removing this liquid from this space for me by the use of his micro-pipette has produced a collapse of the membrane. Allusion has already been made to the fact that polar bodies extruded after the formation of the fertilization membrane may be separated from the egg surface by manipulation and are then free to assume various positions in the space; this could be possible only in liquid but certainly not if the space was occupied by any sort of a "gel."

Professor J. Loeb has shown that the fertilization membrane of echinoderms is impermeable to such colloids as egg white, shark's serum, and even tannic acid. These materials, if added to sea water, cause the thin membrane to crumple onto the surface of the enclosed egg by the osmotic extraction of water from the intervening space; but the membrane regains its original contour when replaced in sea water. This latter fact suggested to Loeb the probability that the space contains some colloid in solution secreted by the egg at the time the fertilization membrane is formed. To this colloid the membrane is impermeable; it therefore exerts its osmotic pressure in excess of that due to the salts of the sea water, serves to raise the membrane from the surface of the egg and to keep it distended.

Experiments by the writer have shown that when fertilized eggs of *Asterias* or *Arbacia* are placed in a two per cent. solution of Witte's peptone in sea water the fertilization membrane crumples instantly as in Loeb's experiments, but that when allowed to remain in the solution the membranes are again distended, indicating that they are slowly permeated by the albumoses in this preparation. Such results can only be obtained where a semi-permeable membrane, one permeable to salts but impermeable to colloids, encloses a space containing a colloidal solution.

4. Still other facts show that the space between the fertilization membrane and the egg surface is filled with a fluid and not with a gel. If *Asterias* eggs are heated to 33° C. they form fertilization membranes in from three to five minutes. By further warming for fifteen minutes they show slow amœboid movements after they have been returned to sea water at 20° C. They may migrate about in the space enclosed by the thin fertilization membrane and up close to that structure at any point. They may throw out long streamer like pseudopodia which meet with no check to their progress anywhere in the space till they reach the thin confining fertilization membrane. This over-heating process may lead to droplet formation and ultimately to partial or complete disintegration of the egg; the débris thus formed is scattered throughout the space and is never held away from the thin fertilization membrane by any gelatinous structure. Similarly if the fertilized eggs of *Asterias* are subjected to the action of diluted sea water they may be caused to swell until each presses tightly against the confining fertilization membrane and completely fills the space. Cytolysis will result in sea water which has been sufficiently diluted and in this case, as in that of cytolysis caused by prolonged warming, the granular material completely fills the perivitelline space which may be more than twice the original diameter of the egg. When extra-ovates rupture through the fertilization membrane after treatment with hypotonic solutions, the egg is drawn over tightly against the inner surface of the membrane at the point of rupture, the thinness of the membrane being evident at the constricted neck between the extra-ovate and the main egg mass.

Again, in the formation of the blastula the cells range up close to the inner surface of the fertilization membrane and completely obliterate the interior space giving a further indication that neither a thickened vitelline membrane nor any "gel" is there.

These demonstrations may be made immediately after fertilization so that there is no necessity to assume that the vitelline membrane or a gel in the perivitelline space has had time to undergo liquefaction.

The above facts and others which might be referred to all indicate the fluid character of the contents of the space between the fertilization membrane and the egg surface and serve to substantiate the conception of the fertilization membrane stated in the opening paragraph of this communication. In the case of both *Arbacia* and *Asterias* the fertilization membrane is a structure which arises at the surface of the egg and is subsequently lifted from it and distended by fluid. Depending upon the time of fertilization of *Asterias* eggs relative to the formation of the polar bodies these structures may be outside the fertilization membrane, inside the perivitelline space or the first outside and the second within the fertilization membrane.

Heilbrunn[1] has reviewed the subject of membrane production by *Arbacia* eggs under the influence of various chemicals. He concludes with Herbst and others that in this form the membrane exists preformed on the surface of the unfertilized egg and that fertilizing agents merely cause its elevation. He gives evidence in support of the view that this elevation is the result of lowering of surface tension and swelling of proteins beneath the membrane. In the light of our findings it is clear that the fluid character of the contents of the intra-membranal space and the progressive increase in its bulk as the membrane is lifted away from the egg indicate that the colloids are in solution and that the process involves the osmotic attraction of water.

It is not yet demonstrated that a membrane exists preformed on the surface of unfertilized *Asterias* egg and the formation of a membrane on a fragment of *Arbacia* egg as described by Moore (*loc. cit.*) are facts which speak, in these instances, for the formation of the fertilization membrane *de novo*.

[1] Heilbrunn, Lewis V., BIOL. BULL., 1913, XXIV, p. 343.